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# Application of Neutron Scattering in Amino Acid Crystals – Structural and Dynamical Information

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## Abstract

In this work results of neutron powder diffraction and inelastic neutron scattering on aliphatic amino acid (L-alanine, L-valine and L-leucine) crystals are presented. The study allows inferring important informations about both the stability of the structures and the dynamics of phase transitions. In particular, it was possible to compare the behavior of different amino acids and to infer that changes in the mean-square displacements observed for two deuterated forms of L-alanine  $\text{ND}_3^+ - \text{C}_2\text{H}_4 - \text{CO}_2^-$  and  $\text{NH}_3^+ - \text{C}_2\text{D}_4 - \text{CO}_2^-$  are due to a structural rearrangement related to thermally activated amplitude reorientation of  $\text{NH}_3^+$  and  $\text{CO}_2^-$ . Conformation changes in L-valine and L-leucine related to the phase transition are also discussed.

**Keywords:** Amino acid crystal, neutron diffraction, inelastic neutron scattering

## 1. Introduction

Amino acids are the basic blocks of proteins of all living beings, but are also found alone in the cytoplasm of several cells. They were produced and selected since the beginning of life in the Earth, although it is not possible to rule out an extraterrestrial origin. Due to the fact that they appear forming zwitterion structure (involving simultaneously the  $\text{NH}_3^+$  and the  $\text{CO}_2^-$  groups) in both aqueous environment and in solid state phase, amino acids have been extensively studied in the last years. The researches deal mainly with spectroscopic and structural properties, giving special attention to the hydrogen bonds between the amino acid and the water molecules in the aqueous solution and among amino acids, when the material is found in crystalline structures [1 – 4].

From the spectroscopic point of view, amino acids have been investigated using mainly Raman spectroscopy [4 – 6], where information about optical phonons is readily obtained. Based on

such studies it has been suggested that amino acid crystals can present structural instabilities at low temperatures or high pressures. Regarding their structural properties, X-rays [7, 8] and neutron scattering diffraction data [5] have been useful in providing a picture of the origin of the stabilities of their crystal structures as well as the role played by the hydrogen bonds.

In particular, it was possible to gain information on the modifications of the hydrogen bonds studying different deuterated amino acids samples using inelastic neutron scattering [9 – 11]. From these data the elastic intensity can be expressed as a function of temperature such as  $I(T) = I(0) \cdot \exp[-Q^2 u(T)^2]$ , where  $Q$  is the scattering vector and  $u(T)$  represents the mean-square displacement of the scattering nuclei. Additionally, if a dynamic transition occurs, one can observe a change in the slope of the observed  $u(T)^2$ . Furthermore, selective deuteration allows tackling changes in the mean-square displacements of particular groups, such as the torsion vibration of  $\text{CH}_3^+$  or the torsion of  $\text{NH}_3^+$  group. Inelastic neutron scattering combined to selective deuteration can also be used to highlight the modes of particular molecular groups by comparing the spectra of the selected deuterated groups [12].

The objective of this chapter is to present a quick review of neutron powder diffraction and inelastic neutron scattering results on various aliphatic amino acid crystals. These investigations allowed to compare the behavior of different aliphatic amino acids (alanine, valine and leucine), in particular, looking at their vibrational and dynamical properties. For L-alanine, for example, combining neutron diffraction to incoherent elastic neutron scattering measurements allowed us to infer that changes in the mean-square displacements observed for two deuterated forms of L-alanine: (i)  $\text{ND}_3^+ - \text{C}_2\text{H}_4 - \text{CO}_2^-$  and (ii)  $\text{NH}_3^+ - \text{C}_2\text{D}_4 - \text{CO}_2^-$  are due to a structural rearrangement related to thermally activated amplitude reorientation of  $\text{NH}_3^+$  and  $\text{CO}_2^-$ .

## 2. Structural and vibrational anomalies observed in selected amino acids

Many results on the vibrational properties of amino acid crystals are known. From these studies one has constructed an interesting picture about the behavior of the crystals in a huge range of temperature and/or pressure. L-alanine, the simplest chiral amino acid, is an example where surprising aspects of the vibrational properties stand. Among these aspects it is possible to cite some: the intriguing step-wise evolution of the wavenumber of some bands [13], the unconventional increasing of the  $c$  lattice parameter when L-alanine is cooled [14], and the localization of vibrational states as revealed by the variation of intensities of the low wavenumber modes at 41 and 49  $\text{cm}^{-1}$  [15]. Simultaneously to the increasing of the  $c$  lattice parameter a progressive conformational change of the  $\text{NH}_3^+$  zwitterion, as well as a strong dynamic Jahn-Teller-like effect due the coupling between  $\text{NH}_3^+$  charge and the lattice were observed [13]. These results can be interpreted as consequence of the N – H stretching self-trapped states related to the N – H...O hydrogen bonds directed along the head-to-tail chains of the zwitterions. According to Ref. [16] these self-trapped states should be associated with some of the anomalies reported for L-alanine. The behavior of the intensity of one lattice mode was realized as consequence of the response of one N – H...O hydrogen bond to respond to the changes in the interaction of the nitrogen and oxygen atoms with other atoms forming different hydrogen

bonds. In fact, it is believed that one can assume that hydrogen bonds in chains of zwitterion have diverse properties from those between the chains and the variation of temperature should produce subtle rearrangement of the hydrogen bond network, explaining the existence of anomalies at low temperature [16]. Under high pressure, L-alanine also shows intriguing aspects. For instance, Raman spectroscopy studies have indicated the occurrence of a phase transition at  $\sim 2.3$  GPa [17], which was later confirmed by X-ray diffraction measurements [18]. A new interpretation, however, has indicated that the modifications about 2 GPa might also be understood as a conformation change of the  $\text{NH}_3^+$  group [19, 20].

The above considerations for the chiral L-alanine can be extended to encompass the racemic material. DL-alanine seems to be stable at low temperature, presenting no evidence of a solid-solid phase transition from room temperature down to 15 K [21]. However, although no modification in the spectral region of the lattice modes – normally between  $30$  and  $200\text{ cm}^{-1}$  – was detected, certain changes in the internal mode region of the spectrum were noted. These changes can be understood as slight conformational modification of the molecule on cooling. In fact, the rocking vibration of  $\text{CO}_2^-$  presents an unconventional behavior, *i.e.*, variation of the wavenumber in step-like shape at specific temperatures. In relation to this, it is important to mention that the data obtained using polarized Raman shows that the main change associated with hydrogen bonds take place along the x-axis direction, instead of the bonds along the z-axis. This behavior was also simultaneously reported in the work presented in Ref. [16] for the chiral partner of alanine crystal.

Crystalline L-leucine, another aliphatic amino acid, presents an interesting behavior when submitted to temperature variation between 300 and 400 K. As shown by Raman spectroscopic measurements, the material undergoes a phase transition at  $\sim 353$  K. One possible explanation for the occurrence of such a modification is the appearance of transverse optical (TO) modes at high temperature. Such a vibrational aspect can be translated to the structural language if one assumes that the room temperature monoclinic structure  $C_2$  change to a monoclinic  $C_s$  structure [22]. In other words, from the point of view of the spectroscopic scrutiny the  $C_2 \rightarrow C_s$  phase transition is a viable path.

Obviously, other questions are relevant as the interaction of amino acid molecules with metal ions. Ions can be present in the living beings as participating of biochemistry processes or nosy hazard agents. Additionally, from a technological point of view it is important to know the influence of metal doping on several crystals with the objective to modify some of their properties, including modification of the crystal habit, thermal conductivity, etc. Analysis of Raman spectroscopy and X-ray diffraction measurements suggested, for example, Ni ions occupy interstitial crystallographic sites in the lattice of L-histidine hydrochloride monohydrate crystal [23]. This specific position is the same occupied by Cu ions in the L-histidine.HCl.H<sub>2</sub>O crystal; the stabilization of the ion – amino acid molecule could be achieved by the interaction between the metal ion and the  $\text{NH}_3^+$  group. On cooling, due to the packing of structure, the interaction ion – amino acid is intensified. To understand the interaction of metal ion and amino acid (representing active part of proteins and peptides) is a task with relevance to biochemistry science.

From this résumé one realizes that many changes might occur when crystalline amino acids are submitted to different pressure and/or temperature conditions. Therefore, one question comes to mind: how the hydrogen bonds connecting the molecules in the solid state phase adapt themselves to the new phases in these amino acids? This question will be addressed in the next sections, where it is explained how neutron diffraction and inelastic neutron scattering experiments can bring unique information to this problem.

### 3. Basic theory

Postulated by Rutherford in 1920 and discovered by J. Chadwick in 1932, neutrons are non-charged subatomic particles with mass rather close to that of the proton  $m_n = 1.675 \cdot 10^{-27}$  kg, magnetic moment equals to  $-1.913 \mu_B$  and a nuclear spin  $s = 1/2$ , which are important and unique properties for developing the theory of neutron scattering [24].

Since neutrons are non-charged particles, their interactions with matter, both nuclear and magnetic, are short range; therefore neutrons can penetrate deeply into matter. This enables us to study the structure and dynamics of materials under very precise environmental conditions. Neutrons can be described using either classical mechanics, with momentum  $\vec{p} = m\vec{v}$ , where  $m$  is the neutron mass and  $\vec{v}$  is its velocity, or quantum mechanics, with momentum  $\vec{p} = \hbar\vec{k}$ , with  $|\vec{k}| = \frac{2\pi}{\lambda}$  defining the wave vector of the neutron and  $\lambda$  its associated wavelength.

This also implies that neutron scattering processes are based on the analysis of momentum and energy transfer, which may occur due to the interactions between neutrons and matter. The momentum transfer vector or the scattering vector,  $\vec{Q}$ , is defined as the vector difference between the incoming and scattered wave vectors as in Eq. (1).

$$\vec{Q} = \vec{k}_i - \vec{k}_f, \quad (1)$$

while the variation in the neutron momentum is given by Eq. (2):

$$\hbar\vec{Q} = \hbar(\vec{k}_i - \vec{k}_f), \quad (2)$$

The corresponding neutron energy  $E$  can be described as described by Eq. (3):

$$E = \frac{p^2}{2m} = \frac{1}{2}mv^2 = \frac{\hbar^2}{2m\lambda} = \frac{\hbar^2 k^2}{2m}, \quad (3)$$

where  $h = 2\pi\hbar = 6.626 \cdot 10^{-34}$  J.s is the Planck's constant. Thus, variation of the energy ( $\hbar\omega$ ) measured in a neutron scattering experiment is given by Eq. (4):

$$\hbar\omega = E_i - E_f = \frac{\hbar^2}{2m} (k_i^2 - k_f^2). \quad (4)$$

During the scattering process, elastic scattering occurs when the variation of energy between neutron and the sample is zero. On the other hand, when this variation is different from zero, the scattering processes is called inelastic.

In a typical neutron experiment the collected information is related to the partial differential cross-section,  $d^2\sigma/(dE d\Omega)$ , given by Eq. (5), according to Ref. [25]:

$$\frac{d^2\sigma}{d\Omega dE_f} = \frac{k_f}{k_i} \frac{1}{\hbar} \left[ b_{coh}^2 S_{coh}(\vec{Q}, \omega) + b_{inc}^2 S_{inc}(\vec{Q}, \omega) \right], \quad (5)$$

where  $S_{coh}(\vec{Q}, \omega)$  and  $S_{inc}(\vec{Q}, \omega)$  are the so-called coherent and incoherent scattering functions. These functions are Fourier transformations of the van Hove correlation functions  $G(\vec{r}, t)$ , which can be described as the probability for an atom at the origin at time 0, to also be found within the unit volume at a position  $\vec{r}$  at a time  $t$ .

The probability of interactions between the neutrons and the atoms is a characteristic of each isotope and described by two terms: coherent and incoherent cross sections ( $\sigma_{coh}$  and  $\sigma_{inc}$ ), as shown in Table 1. The cross section is related to the coherent ( $b_{coh}$ ) and incoherent ( $b_{inc}$ ) scattering length by the following relation:  $\sigma = 4\pi b^2$ . The high  $\sigma_{inc}$  value of  $^1\text{H}$  in comparison to the other elements allows the assignment of the vibrations related to hydrogen atoms. On the other, the high value of the coherent cross section of  $^2\text{D}$  when compared to  $^1\text{H}$  allows to locate the hydrogen atom by using deuteration.

	$^1\text{H}$	$^2\text{D}$	C	O	N	Cl
$\sigma_{coh}$	1.76	5.59	5.55	4.23	11.0	11.5
$\sigma_{inc}$	80.27	2.05	<0.01	<0.01	0.50	5.30

**Table 1.** Coherent ( $\sigma_{coh}$ ) and incoherent ( $\sigma_{inc}$ ) cross-sections in  $10^{-24} \text{ cm}^2$  for selected elements and isotopes [26].

For crystalline matrices, the coherent scattering function in Eq. (5) is written as given in Eq. (6):

$$S_{coh} = \left| F(\vec{Q}) \right|^2 \delta(\omega) \sum_{\vec{G}} \delta(\vec{Q} - \vec{G}) + \sum_{s, \vec{q}} \left| F(s, \vec{q}) \right|^2 \frac{\left\langle n(\omega) + \frac{1}{2} \pm \frac{1}{2} \right\rangle}{\omega} \delta(\omega \mp \omega_{s, \vec{q}}) \sum_{\vec{G}} \delta(\vec{Q} + \vec{q} - \vec{G}), \quad (6)$$

where the first term represents Bragg scattering and in a diffraction experiment the Bragg's Law is satisfied:

$$n\lambda = 2d \sin \theta, \quad (7)$$

here  $n$  is a positive integer and  $\lambda$  is the wavelength of incident wave and the angle  $\theta$  is defined as the angle between the incoming ray and the diffraction atomic plane.

The second term in Eq. (6) describes collective atomic motions (phonons), where  $\vec{q}$  is the wave vector of the collective atomic motion and  $s$  an index that specifies the various modes of such collective motions.

Contributions from single particle motion are described by  $S_{inc}(\vec{Q}, \omega)$  as given by Eq. (8):

$$S_{inc}(\vec{Q}, \omega) = S_{inc}^{trans}(\vec{Q}, \omega) \otimes S_{inc}^{rot}(\vec{Q}, \omega) \otimes S_{inc}^{vib}(\vec{Q}, \omega), \quad (8)$$

In Eq. (8) the terms describe contributions from the translational, rotational and vibrational modes, respectively.

## 4. Experimental details

In order to obtain the results presented in this chapter, a series of facilities were utilized. Neutron powder diffraction (NPD) experiments were carried out using the powder diffractometers E9 located at the Helmholtz-Zentrum Berlin für Materialien und Energie (Berlin, Germany) and D2b at the Institut Laue-Langevin (Grenoble, France). Such equipments allowed the determination and refinement of crystal structures with very high resolution, furnishing lattice constants and internal atomic coordinates with great precision. Through the experiments with temperature variation the data were analyzed using a crystallographic model from Ref. [27] Due to the thermal diffuse scattering contribution, attention was paid to the modeling of the background.

Incoherent elastic neutron scattering measurements were carried out using the time-of-flight spectrometer NEAT at the Helmholtz-Zentrum Berlin für Materialien und Energie as well as the backscattering instrument IN10 located at the Institut Laue-Langevin.

The analysis of the evolution of the elastic line centered around  $E = 0$ , (energy resolution,  $\Delta E = 100 \mu\text{eV}$  (NEAT) or  $1 \mu\text{eV}$  (IN10)) as a function of temperature gives information about the mean square displacement of the atoms around their equilibrium positions or, in very broad terms, describes the crystal flexibility. On the other hand, the inelastic part of the spectra gives information on the lattice phonons as well as on the inter-molecular vibrations of the samples. The obtained information is complementary to infrared (IR) and Raman scattering (RS).

## 5. Looking inside amino acid crystals

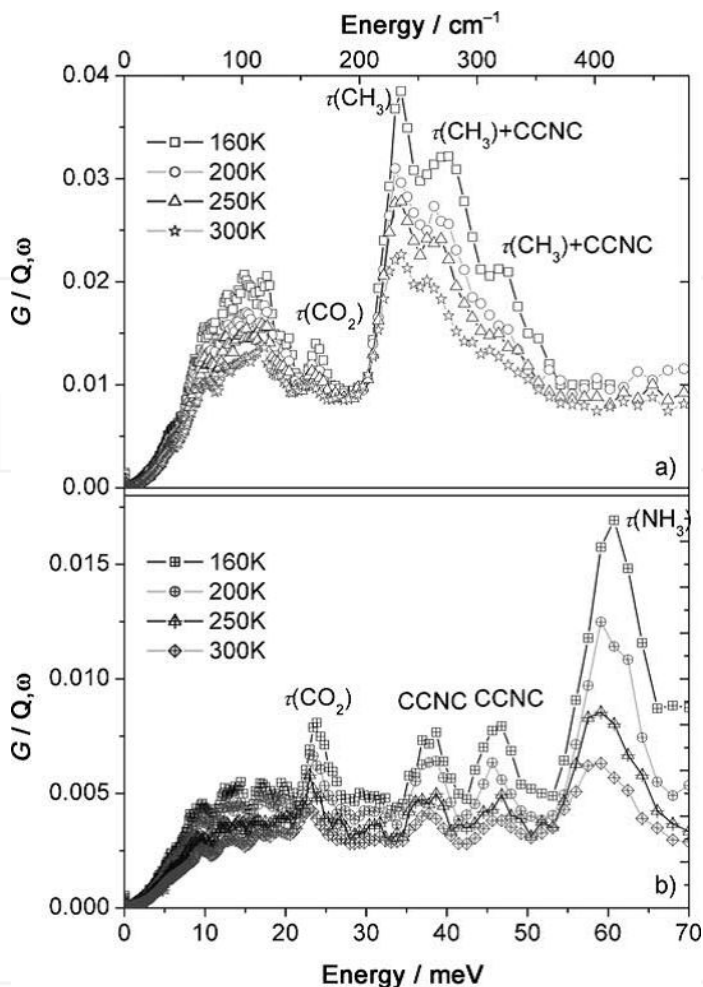
In relation to amino acid crystals there is a pivotal point to be considered when using neutron spectroscopy or neutron diffraction: the hydrogen bonds that connect the various molecules together in the crystal structure. In this chapter results on crystals of the amino acids L-alanine, L-valine and L-leucine are discussed.

The first discussion will furnish a picture on the simplest chiral amino acid, L-alanine. Selective deuteration of L-alanine were analyzed: (i)  $\text{C}_2\text{H}_4\text{-(ND}_3^+\text{)-CO}_2^-$ , Ala-ND3; (ii)  $\text{C}_2\text{D}_4\text{-(NH}_3^+\text{)-CO}_2^-$ , Ala-CD4; (iii)  $\text{C}_2\text{D}_4\text{-(ND}_3^+\text{)-CO}_2^-$ , Ala-D7. L-alanine crystallizes in an orthorhombic structure with four molecules per unit cell in a  $P2_12_12_1$  space group. The molecules are distributed spatially as head-to-tail chains along the crystallographic  $c$ -axis, presenting a complex three dimensional network of hydrogen bonds. Here we recall that the  $c$ -axis a stepwise change of the size parameter on cooling. Combining inelastic neutron scattering (INS) and neutron powder diffraction (NPD) is possible to study the relationship between the local dynamics of  $\text{NH}_3$ ,  $\text{CO}_2$  and  $\text{CH}_3$  and to gain insights about the behavior of hydrogen bonds.

Figure 1 presents the inelastic neutron scattering spectra of (a) Ala-ND3 and (b) Ala-CD4 for several temperatures in the energy range from 0 to 70 meV. Data were obtained using the NEAT spectrometer. It is possible to observe several normal modes: lattice modes below 20 meV and other modes such as torsion of  $\text{CO}_2$  group,  $\tau(\text{CO}_2^-)$ , torsion of  $\text{NH}_3$ ,  $\tau(\text{NH}_3^+)$ , and torsion of  $\text{CH}_3$ ,  $\tau(\text{CH}_3)$ , as specified in the figure. It is interesting to observe the peak located  $\sim 60$  meV that is associated with  $\tau(\text{NH}_3^+)$ . The width of this peak can be understood as consequence of the anharmonicity of the hydrogen bonds because all three protons participate of N-H...O hydrogen bonds. The influence of anharmonicity is not restrict to the torsion of the ammonia group; as it can be noted in Figure 1 the large peaks associated with the lattice modes that involves bending and stretching of hydrogen bonds can be ascribed to the same effect. Additionally, if one plots the energy of  $\tau(\text{CO}_2^-)$  mode in Ala-ND3 (not shown in Figure 1) as a function of temperature, one notes a discontinuity between 150 and 200 K. Such a discontinuity coincides with a discontinuity of the wavenumber of a band associated with  $\tau(\text{CO}_2^-)$  in the Raman spectrum at temperatures in the same interval.

Figure 2 presents the neutron diffraction patterns of Ala-CD4 for two different temperatures. The measurements were obtained with a neutron wavelength of 2.8060 Å. Impressively, at  $T = 100$  K, it is observed a Bragg peak at  $\sim 2\theta = 78^\circ$  that does not belong to the  $P2_12_12_1$  orthorhombic structure. In the attempt to index this peak authors were guided to assume a primitive orthorhombic cell with the  $a$ -axis with double size [11]. It is remembered that the non-deuterated L-alanine presents any strong modification in its lattice parameter, although the crystal itself shows a series of anomalies at low temperatures. As a consequence, the result presented in Figure 2 suggests that the partial deuteration of L-alanine induces a solid-solid phase transition. Interestingly such a phase transition can be seen as an enhancement of an anomaly observed in the non-deuterated partner at  $T \sim 260$  K [13]. The doubling of the  $a$ -lattice parameter involves changes in the two hydrogen bonds represented in Figure 3 and a rearrangement of the whole molecule. Between 270 and 250 K, a decreasing in the D(2)...O(2)

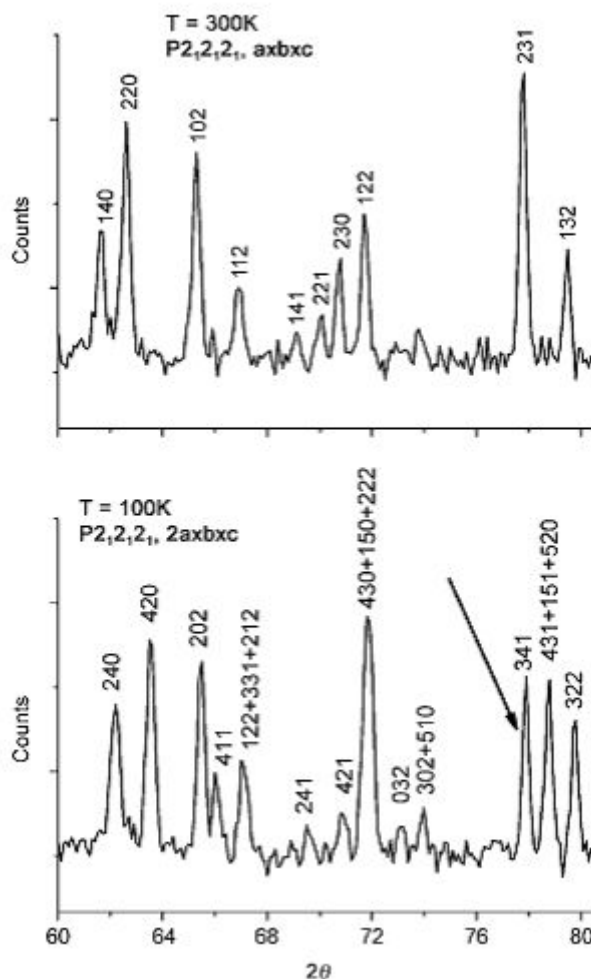




**Figure 1.** Inelastic neutron scattering spectra at various temperatures for (a) Ala-ND<sub>3</sub> and (b) Ala-CD<sub>4</sub>. The assignment of the main modes is given [11].

bond is observed and an impressive anomaly between 250 and 100 K. On the other hand, the D(1)...O(1) bond becomes longer when temperature is lower than 100 K.

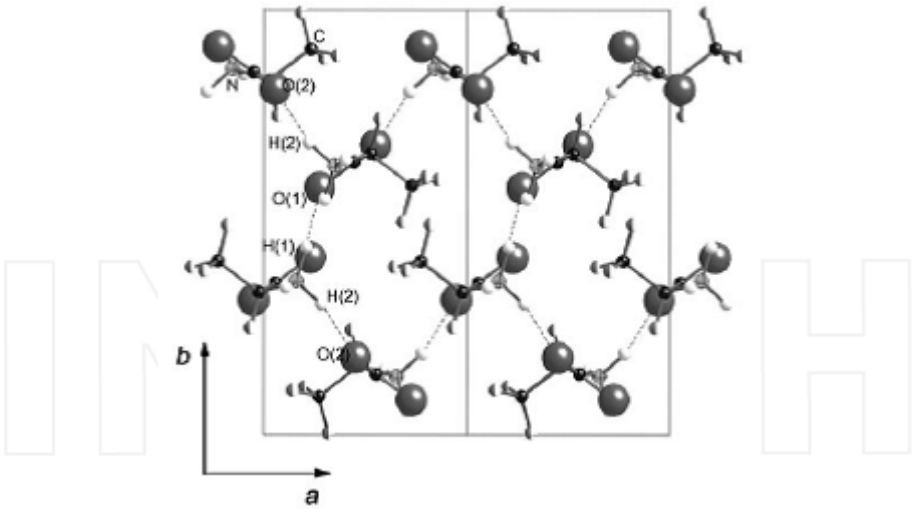
Figure 4 presents  $\langle u_{\text{H}}(T)^2 \rangle$  of  $\text{C}_2\text{H}_4\text{-(ND}_3^+\text{)-CO}_2^-$  and  $\text{C}_2\text{D}_4\text{-(NH}_3^+\text{)-CO}_2^-$ , where  $u_{\text{H}}(T)$  represents the mean-square displacement of the scattering nuclei. Here, as specified previously,  $I(T) = I(0) \cdot \exp[-Q^2 u(T)^2]$  is the elastic intensity expressed as a function of temperature (the temperature dependence is embedded in  $u_{\text{H}}$ ). In fact, once the elastic intensity has been experimentally determined, one obtains  $u(T)^2$ . As it is expected, due to the large incoherent cross section of the hydrogen,  $u(T)$  is supposed to originate only from the motion of H atoms. Another aspect to be



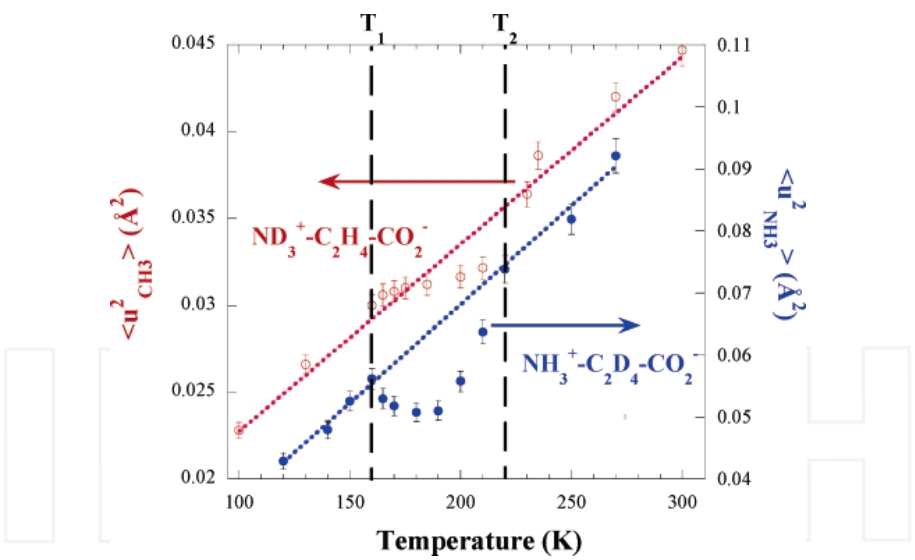
**Figure 2.** Neutron diffraction patterns of  $C_2D_4-(NH_3^+)CO_2$  at  $T=300\text{ K}$  and  $T=100\text{ K}$  measured with the E9 diffractometer at Hahn-Meitner-Institut. The arrow indicates the peak that could not be indexed with the original  $P2_12_12_1$  structure [11].

considered is the following: change in the slope of  $u(T)^2$  indicates a dynamical transition in the material. From the Figure 4 one observe that at 160 K and at 220 K there are clear changes in the slop, that can be interpreted as a phase transition or, at least, to a structural rearrangement. Such rearrangement should be probably related to thermally activated large-amplitude reorientations of both  $CH_3$  and  $NH_3$  smithereens of the L-alanine molecule.

The previous paragraphs showed that in the study of partially deuterated L-alanine crystal, the  $H(1)...O(1)$  hydrogen bond length increases below 100 K on decreasing temperature and



**Figure 3.** Representation of the crystal structure of L-alanine showing the *ab*-plane. The two hydrogen bonds modifies at low temperature are shown [11].



**Figure 4.** Mean-square dependence from the normalized elastic intensity as a function of temperature. The left scale and open circles represent data for  $C_2H_4-(ND_3^+)-CO_2^-$  and the right scale and full circles represent data for  $C_2D_4-(NH_3^+)-CO_2^-$  [10].

the H(2)...O(2) length presents an anomaly between 100 and 250 K. What about the behavior of fully deuterated L-alanine under the scrutiny of INS and NPD techniques? Roughly

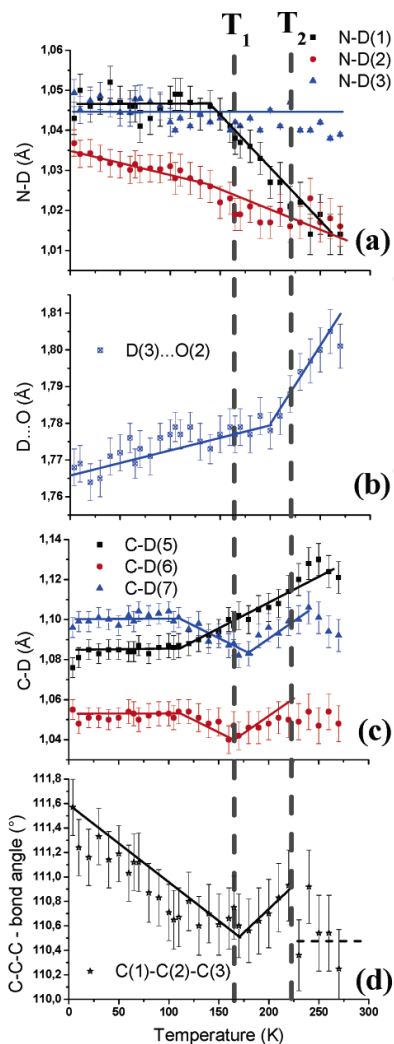
speaking, deuteration, or isotopic substitution,  $D \rightarrow H$ , produces strong modification in the length of the hydrogen bond. This change generally affects the tunneling rates and the motional properties of the molecules, an effect known as Ubbelohde effect; it is observed in crystals with complex network of hydrogen bonds and even in isolated pair of this type of bond.

Figure 5 presents the temperature dependence of several parameters related to the hydrogen bonds for the fully deuterated L-alanine crystal,  $C_2D_4-(ND_3^+)-CO_2^-$ , Ala-D7. Several anomalies are described in the next paragraphs.

From the measurement it is possible to realize that the N-D(3) distance remains almost constant between 10 and 270 K. On the other hand, the N-D(1) distance remains constant only between 10 and 130 K, and diminishes for temperatures higher than 130 K (Figure 5a). Also interesting is the dramatic increase of the D(3)...O(2) hydrogen bond responsible for connecting the molecules into columns (see Figure 5b). This last point is in complete accordance with the fact that torsion of  $NH_3$  group presents important anharmonicity, as depicted during the discussion of Figure 1. So, one can understand the anharmonicity of  $\tau(NH_3^+)$  as consequence of the modification of one specific hydrogen bond during the temperature variation [10]. Looking at the average C-D lengths one notes anomalies at low temperatures as presented in Figure 5c. From the Figure 5d one also notes an anomaly in the C-C-C bond angle that extends from about 160 K up to 225 K. In this point is important to emphasize that Raman spectroscopy studies showed splitting of bands associated with lattice modes at  $T \sim 170$  K. This means evidence of a phase transition. Additionally, it was observed anomalies in the Debye-Waller factor, confirming the phase transition at about 170 K in the fully deuterated L-alanine [10].

L-valine was the second aliphatic amino acid crystal studied through neutron techniques. Investigations so performed revealed aspects related to hydrogen bonds, ultimately, responsible for the structural stability of the material. L-valine crystallizes in a monoclinic structure with  $P2_1$  space group and four molecules per unit cell. From these molecules, two assumes *gauche I* conformation and two others *trans* conformation. Previous work using Raman spectroscopy suggested L-valine should undergo a solid-solid phase transition because impressive modifications of the bands associated with lattice modes were observed at low temperatures [28].

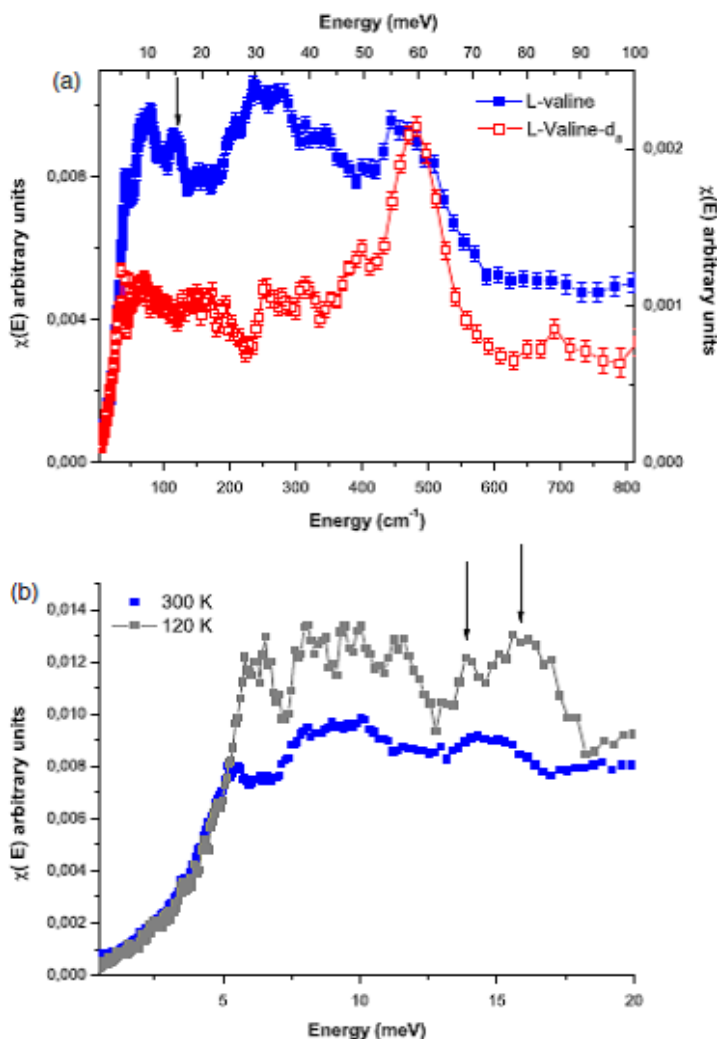
Figure 6 presents the dynamical susceptibility ( $\chi''(E)$ ) of both L-valine hydrogenated  $[(CH_3)_2CHCH(NH_2)CO_2H]$  and deuterated  $[(CD_3)_2CDCH(NH_2)CO_2H]$  (synthesized by Dr. Ehrenstorfer GmbH from Augsburg, Germany). The comparison of the INS spectra of L-valine and deuterated L-valine (L-valine- $d_8$ ) shows differences that can be understood as follows. At first, an intense band at  $480\text{ cm}^{-1}$  in the spectrum of L-valine- $d_8$  points to the accuracy of the assignment of the vibrations involving the N-H...O hydrogen bonds. Also, a decrease of the signal for energy lower than  $450\text{ cm}^{-1}$  indicates they are associated with the  $CH_3$  and  $CO_2$  groups and the N-C-C units [9]. It is impressive in the room temperature INS spectrum of L-valine the presence of a mode at  $\sim 120\text{ cm}^{-1}$ . It is remembered that such a band not visible at  $T = 300\text{ K}$  appeared in the Raman spectra of L-valine for temperatures lower than 120 K. In other words, this band could be associated with a mode not active in the Raman spectrum of the monoclinic room temperature structure, being active only in the infrared. During the phase transition the mode should become Raman active and the phase transition should be realized as consequence



**Figure 5.** Temperature dependence of (a) N-D, (b) D...O, (c) average C-D length, (d) average C-C-C bond angles for fully deuterated L-alanine. The molecule is represented in the top left side of the figure and dashed lines are guides to the eyes [10].

of the activation of an infrared mode. An additional aspect of the study is to show (Figure 6b) the splitting of the band at  $\sim 15$  meV at low temperature when compared with the spectrum recorded at 300 K, reinforcing the idea of a phase transition, as supported by the Raman spectroscopic study [28].

Up to now the present authors have seen several aspects of the bonds on L-alanine and an explanation for the phase transition undergone by L-valine using neutrons. It can be noted

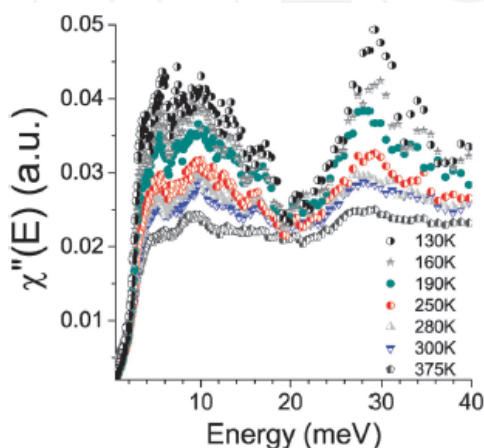


**Figure 6.** (a) Dynamical susceptibility [ $\chi''(E)$ ] for both L-valine (full squares) and L-valine- $\text{d}_5$  (open squares) using a 5.1 Å incident wavelength at the NEAT spectrometer. (b)  $\chi''(E)$  of L-valine for two temperatures, 300 K and 120 K. The arrow in part (a) corresponds to the mode at 120  $\text{cm}^{-1}$ , observed in the low temperature range by Raman spectroscopy [12].

that subtle conformational change can lead to impressive modifications in the structural and vibrational properties of these two members of the amino acid family. Now, the discussion deals about another aliphatic amino acid. L-leucine,  $\text{NH}_3\text{-CH-CO}_2\text{-CH}_2\text{-CH}(\text{CH}_3)_2$ , crystallizes in a monoclinic structure  $P2_1$  with four molecules per unit cell. The carboxyl and amino groups are hydrogen bonded in a double layer, forming the crystal structure. The van der Waals interaction plays its role by connecting neighboring layers [1]. Due the existence of an addi-

tional  $\text{CH}_2$  group in its chain, L-leucine is slightly more hydrophobic than L-valine. A previous research demonstrated the occurrence of a phase transition in L-leucine at  $\sim 353$  K possibly maintaining the crystal with a monoclinic structure [22].

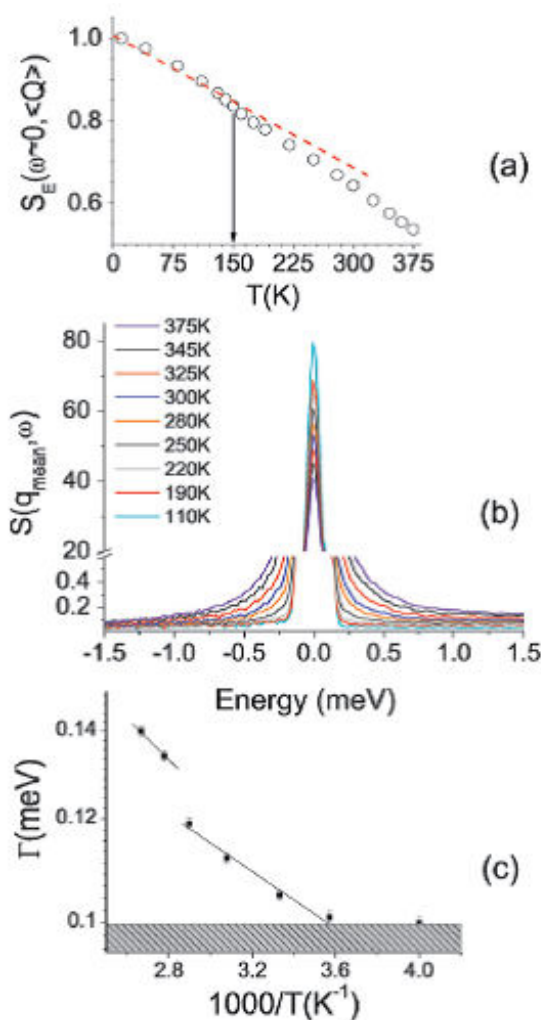
Figure 7 presents the dynamical susceptibility function  $\chi''$  obtained from calculations based on the inelastic data from NEAT. It is perceived that below 280 K the mode at 30 meV (approximately at  $240\text{ cm}^{-1}$ ) presents a red shift down to 190 K and below this, presents a blue shift; also, the intensity increases on cooling. Interesting enough is the occurrence of a redistribution of intensity of the bands between 300 and 375 K that can be correlated with the phase transition observed at 353 K. The phase transition can also be glimpsed by the intensities of most modes located below 30 meV, associated with lattice modes, that show pronounced changes in the interval 300 – 375 K.



**Figure 7.** (a) Dynamical susceptibility [ $\chi''(E)$ ] for L-leucine in the temperature range from 130 and 375 K measured in the energy interval from 1 to 30 meV. The mode at  $\sim 30$  meV is associated with an out-of-plane vibration of  $\text{CH}_2$  [29].

Supposing  $S(Q, \omega)$  the scattering function, with  $Q$  being the magnitude of the scattering wave vector and  $\omega$  being the energy transfer, it is possible to decompose it in three different components:  $S_E(Q, \omega = 0)$ , the elastic;  $S_E(Q, \omega \sim 0)$ , quasi-elastic;  $S_E(Q, \omega > 0)$ , inelastic. The study of the elastic scattering decay can furnish information about transitions because it appears as changes in the temperature dependence of the elastic intensity. Figure 8a shows the elastically scattered intensity as a function of temperature for L-leucine. The main contribution to  $S_E$  comes from the hydrogen atoms. Within the time scale of  $\sim 10$  ps originated from the resolution of the equipment one can argue that the transition at  $\sim 150$  K is a consequence of  $\text{CH}_3$  groups. Such an anomaly confirms differential scanning calorimetry (DSC), as well as X-ray diffraction measurements [29].

In addition, one notes the broadening of the quasi-elastic response apparently disappears in the temperature range 220 – 250 K and the peak height decreases. It was possible to derive a



**Figure 8.** (a) Elastically scattered intensity as a function of temperature for L-leucine. An anomaly in the temperature evolution is marked by an arrow. (b) Dynamic structure factors obtained for  $AE = 98 \mu\text{eV}$  for several temperatures averaged over the whole range of  $Q$ . (c) Lorentzian half width at half maximum (HWHM),  $\Gamma$ , obtained using an Arrhenius-like relation [29].

qualitative description by fitting the quasi-elastic  $S_{QE}$  using a Dirac function and a Lorentzian linewidth, which could describe, respectively, the elastic and quasi-elastic signals. In this approach it is possible to correlate the quasi-elastic broadening ( $\Gamma$ ) with the activation energy,  $E_{act}$ , by the relation  $\Gamma = \Gamma_0 \cdot \exp[-E_{act}/kT]$ . The most important point derived from this relation is presented in Figure 8c, where a discontinuity, associated with the high temperature phase transition is observed between 345 and 360 K.



## 6. Conclusions

Neutron diffraction and inelastic neutron scattering are powerful techniques to investigate several materials, among them, amino acid crystals, as it was shown in this chapter. From these studies it was presented the possibility to correlate small variations of the network of hydrogen bonds to conformation changes in the amino acid molecules.

L-leucine, that presents only one polymorph at room temperature, was shown to undergo phase transitions at low and high temperatures. The new phases were analyzed and insights about the conformation of the molecules were attained.

In the case of L-valine it was possible through comparison between Raman spectroscopy and inelastic neutron scattering to identify infrared active modes responsible for the phase transition undergone by the crystal at approximately 120 K.

Finally, a series of information was obtained for the simplest proteic amino acid crystal, L-alanine. In the case of the fully deuterated sample, L-alanine-d<sub>7</sub>, our results showed a clear evidence of a structural phase transition at ~ 170 K. Such fact can be related to the Ubbelohde effect, or in other words different dimensions of the hydrogen bonds stabilize differently the deuterated and the non-deuterated crystals. Consequently, although the deuterated sister presents a phase transition, the non-deuterated structure, even if a series of structural and vibrational anomalies are observed, is stable at low temperatures. Our investigations point to the fact that the Ubbelohde effect can be significant, even if only small changes of hydrogen bond dimensions occur.

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